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Symptotic detection of chimerism

Y does it matter?

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Microchimerism (MC), transplacental acquisition of allogeneic cells from the mother (maternofetal MC) or from the fetus (fetomaternal MC) has been in the focus of research recently. Amplicons using Y-chromosome specific SRY and DYS14 sequences have been used as markers to trace cells from a male fetus in the mother. The sensitivity of these markers in formaldehyde fixed paraffin embedded samples, however, is less than optimal. To study chimerism in breast cancer we took advantage of the evolutionary history of the Y chromosome and designed amplicons on gene repeats to generate additive PCR signals. The increased sensitivity detected high incidence of male chimerism in normal breast tissues. We also showed correlation with protection from cancer with unique quantitative biology. Accumulating data from biology and medicine indicate that natural chimerism is astonishingly frequent and may affect human conditions. We hypothesize that it has significant evolutionary ramifications as well.

Keywords: chimerism, microchimerism, PCR, quantitative, symptotic, breast, cancer, evolution

Abbreviations: MSY, male specific region on the Y chromosome; MC, microchimerism; SRY gene, sex-determining region Y; TSPY gene, Testis-specific Y-encoded protein; DYS14, marker on the Y chromosome; FFPE, formaldehyde fixed paraffin embedded; OCT4 gene, octamer-binding transcription factor 4 (also known as POU5F1: POU domain, class 5, transcription factor 1);

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The predicament of male biology is well known in reproductive genetics: the Y-chromosome is heading for extinction. About 166 million years ago, one of the X chromosomes began diverging from its pair to become the ancient Y chromosome in eutherian males.[1] Autosomal chromosomes (and the X in females) are diploid, so homology-based repair can use the code in the second copy. Without a backup copy, however, repair of the Y chromosome was not possible. As genetic losses could not be replaced, the Y became gradually smaller. At present, the human Y codes for only about 3% of its original male specific gene set,[2] and its future is far from secure. There are in fact species that have lost the Y altogether[3,4] and must scramble to maintain sex for its undeniable rewards, genetically speaking.

With an unexpected turn in its uphill battle, however, the Y started fighting back. Although there is still no copy of its sequences outside (the non-pseudoautosomal sequences), it started depositing its own copies within its ampliconic component.[5] Using these copies the Y can now replace lost sequences internally, which appears to be a winning strategy. In the last 25 million years only one gene out of 20 has been lost in the male specific regions (MSY)[2] and the Y seems to be stabilized in its diminutive form. Interestingly, it appears to be a blessing for mankind. However small, the Y became a hot spot of human evolution with 30% divergence from the corresponding chimpanzee sequence,[6] in contrast to <1% overall genomic differences.[7]

The extant Y has also proved a blessing in a more limited scope, in the growing field of microchimerism studies (MC). Natural acquisition of allogeneic cells through transplacental mechanisms from the mother (maternofetal MC) and from the fetus (fetomaternal MC) have been well documented. The Y chromosome is an obvious marker to detect cells from the male fetus in the female host. Various Y markers have been used in chimerism studies. The SRY gene product is key to induce male development and its absolute male specificity supports

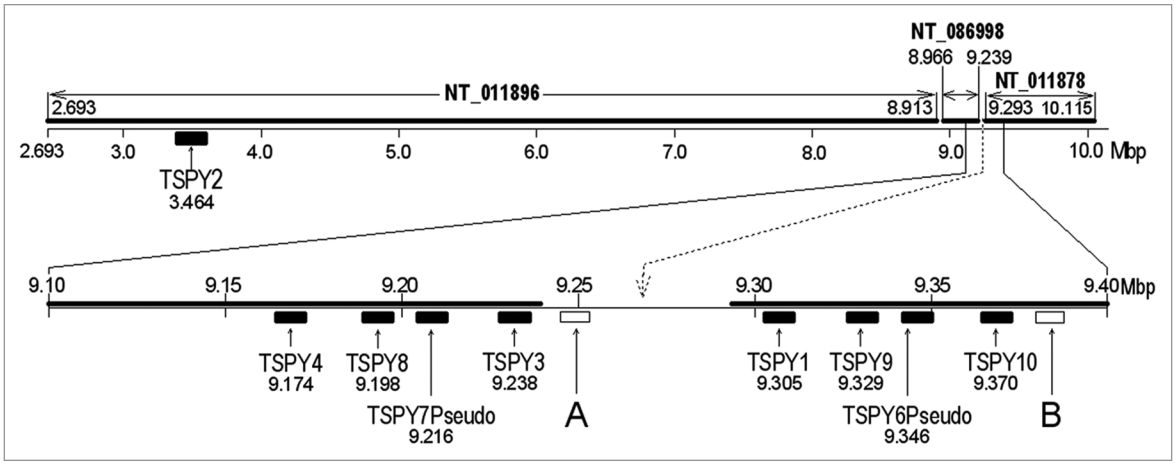

Figure 1. Map positions of the 9 TSPY genes of the symptotic amplicon. The map depicts the TSPY gene positions (filled rectangular boxes). The three contigs are in bold above the lines. Y-chromosomal positions are indicated below the line and below the genes, in million base pairs (Mbp). The two empty rectangles (**A and B**) are low homology TSPY sequences (no contribution to the symptotic signal). Note that, speculatively, the genomic localizations reflect at least 3 duplication events: first, TSPY1 → TSPY9; second, TSPY1 + 9 → TSPY6p + 10; third, the TSPY1+9+6p+10 unit → TSPY4+8+7p+3 unit. The origin of TSPY2 is not clear.

Y-specific amplicons.[8] Another frequently used marker is the DYS14 sequence[9] that overlaps with the first exon/intron boundary of several TSPY gene repeats. The available DYS14 PCR protocols amplify one or at most three of the ~35 TSPY repeats (GenBank) as their designs target highly diverged intronic sequences. These PCR markers, therefore, target low copy number sequences on the Y, but still give specific results using sufficient amounts of undegraded DNA.

That is not the case, however, with formaldehyde fixed paraffin embedded (FFPE) tissues. To assess male chimerism in breast cancer we attempted to use DYS14 and SRY markers on FFPE sections, but quantitative measurements were not possible. To find a way to increase sensitivity, we realized that the evolutionary stabilization of the Y through internal reduplications offered a unique possibility. By selecting reiterated amplicons flanked by conserved sequences for primers, we could increase the output without increasing the input and/or quality of the template.

We ran GenBank homology searches with Clustalw analysis using MSY genes and found nine iterations of an amplicon within the TSBY family (**Fig. 1**). As the primer sequences were conserved throughout (at least in the 3′ seeding regions), and thus all products contributed to a common amplicon (they all "fall together"), we decided to call the approach "symptotic," meaning the same. For quantitative analysis, we worked out a normalization amplicon with eight symptotic templates using the OCT4 gene, an ancient sequence with several remnant pseudogenes from its long history.[10] The primer sequences and the symptotic amplification protocols have been published.[11]

Our data showed high incidence of male DNA in normal women, 56% of breast tissue samples were positive. We also found interesting correlations with breast cancer. Allogeneic presence correlates with protection from breast cancer, but the effect is dose dependent. Chimerism both below and above a particular level correlated with cancer; in between, however, at levels that corresponded with endogeneous stem/progenitor densities in the mammary gland, male allogeneic cells were protective. The implications are far reaching and imply a potential niche-competition mechanism in chimeric stem cell biology.

In evolutionary terms, the implications are even more significant. The data strongly suggest that chimerism is a relevant biological phenomenon and probably the result of a relatively recent evolutionary coincidence. In primitive mammals (monotremes, marsupials) the placenta is short-lived and insignificant. Even in more advanced eutherians the placenta has insulating layers (epithelio/endotheliochorial) and does not invade the myometrium. In primates and particularly in humans, however, evolution was presented with a quandary: increasing oxygen demand to develop an enormous brain, but with decreasing pelvic circulation imposed by bipedalism[12] and by limits on maternal metabolism.[13] To supply the fetus, humans developed an almost parasitic, deeply invasive hemochorial placenta that reaches the inner third of the myometrium, a feature shared only by great apes.[12] By coincidence, however, the barriers were also eliminated between two genetically different biological organisms.

The resulting massive exchange of allogeneic pluripotent stem cells is a relatively recent evolutionary experiment. The last common ancestor of extant primates lived ca. 8–10 million years ago. This is an extremely short time to see how evolution sorts out these new waves of stem cells. The fact that chimerism correlates with both positive and negative biological consequences suggests that evolution is in the middle of this process.

In summary, it is a rare opportunity to witness evolution in action. But the rarest of all is to witness the unfolding of an entirely new evolutionary trend. For billions of years, the primary concept of life was to maintain genetic integrity at any price. We know of only two exceptions: the

endosymbiotic fusion of mitochondria/chloroplasts, and genetic fusion by sexual reproduction. The long-term massive integration of allogeneic stem cells at the organismic level may be a third. It appears to be uniquely extensive in primates and may be part of the puzzling stellar evolution of one of them, considering that the brain is a major target for allogeneic integration.[14] Altogether, what we may witness is potentially a breakdown of a primary concept in biology, a new avenue in evolution, and that is something worth watching.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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